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| <b>(54) Title:</b> METHOD AND DEVICE FOR DETERMINATION OF PROTEINS EMPLOYING ANTIGEN/ANTIBODY REACTIONS<br><br><b>(57) Abstract</b><br><p>A body sample to be analyzed is passed through a series of laminated membranes which may be considered as two separate membrane units. The first unit comprises a separator membrane superimposed in capillary contact with a reactor membrane. The second unit comprises a collector membrane superimposed on a capture membrane or as an extension of the capture membrane. Initially the units are not in contact with each other, but at the appropriate time they are brought into contact. Selected antibodies are appropriately distributed throughout the membrane structure. In a preferred embodiment, the devices of the present invention are used for the separation of low density lipoproteins from whole blood.</p> |           |   |

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# METHOD AND DEVICE FOR DETERMINATION OF PROTEINS EMPLOYING ANTIGEN/ANTIBODY REACTIONS

## FIELD OF THE INVENTION

This invention relates to a methods and devices for the separation of selected  
5 proteins from body fluids employing antigen/antibody reactions. In a preferred  
embodiment, the devices of the present invention ar used for the separation of low  
density lipoproteins from whole blood.

## BACKGROUND OF THE INVENTION

10 Elevated plasma cholesterol and triglycerides levels are associated with increased  
risk for coronary disease. Among cholesterol containing lipoproteins in plasma,  
elevated levels of low density lipoproteins (LDL) and decreased levels of high  
density lipoproteins (HDL) are described to be independent risk factors for  
premature coronary heart disease (CHD).

15 The National Cholesterol Education Program (NCEP) guidelines recommend the  
identification of individuals at risk for CHD by plasma cholesterol measurements  
and suggest a direct LDL cholesterol test for more accurate assessment and  
management of patients at risk. Primary criterion for treatment decisions require  
means for differentiation and accurate measurement of different lipoproteins'  
cholesterol levels. The three methods most frequently used to estimate LDL are  
20 the ultracentrifugation method, the Friedewald formula method and the antibodies  
methods. In the following discussion, the term LDL refers to a group of  
heterogeneous particles (buoyant density greater than 1.019 Kg/L but less than or  
equal to 1.063 Kg/L) comprised of LDL+IDL (intermediate density lipoprotein or  
VLDL remnants) + Lp(a) fractions.

25 The ultracentrifugation method for LDL cholesterol is based upon density  
differential centrifugation of serum or plasma for 18 hours at 109,000 x g and  
isolation of 1.006 Kg/l fraction (chylomicron and very low density lipoprotein.

VLDL) and 1.006 Kg/1 infranate (LDL and HDL fractions). Cholesterol is then measured in 1.006 Kg/1 infranate before and after precipitation of LDL by the addition of dextran-Mn or heparin-Mn. LDL cholesterol is calculated as follows:

$$\text{LDL Chol.} = 1.006 \text{ Kg/1 infranate Chol minus HDL Chol. (LDL precipitated by dextran-Mn or heparin-Mn).}$$

Such method is both time consuming and labor intensive and is not available in most clinical laboratories.

Friedewald Formula method which is well known and understood is most commonly used by clinical laboratories and is based upon determination of total cholesterol in serum before and after precipitation of VLDL and LDL fractions by dextran-Mn (HDL remains in solution) and estimation of VLDL cholesterol by measuring total fasting serum triglyceride (TG) levels. LDL chol. is then estimated as follows:

$$\begin{aligned} \text{LDL Chol.} &= \text{Total Chol. minus (HDL Chol + VLDL estimate)} \\ \text{VLDL Chol. estimate} &= \text{TG levels/5 ... mg/dl or} \\ &\text{Tg levels/2.22 mmol/l} \end{aligned}$$

There are a number of problems associated with the use of this method. Samples must be from fasting patients. The indirect estimation of VLDL Chol. is invalid if the TG levels are high *i.e.*, above 400 mg/dL. Even though the method does not require ultracentrifugation, it involves multiple assays and steps which result in increased variability in the measurements.

The direct measurements of LDL with antibodies is based upon selective retention of VLDL and HDL lipoproteins in plasma or serum and quantification of LDL in the effluent using dry chemistry methods employing antigen/antibody reactions which can be visualized. The measurement of LDL cholesterol is effected as follows:

$$\text{LDL Chol} = \text{plasma or serum Chol. (after removal of VLDL \& HDL fractions).}$$

The only other technique that provides measurement of LDL directly is based upon specific removal of lipoprotein other than LDL from serum/plasma by affinity purified goat polyclonal antisera coated to latex particles. In the technique, serum or plasma is obtained by routine laboratory techniques and added to a small cup containing immunoseparation reagents. After brief incubation followed by centrifugation, LDL is recovered and quantitated in the filtrate.

While such a method can be used with non-fasting patients, it still requires initial centrifugation to obtain plasma or serum followed by another centrifugation to isolate LDL fraction.

- 10 As presently contemplated, the methods and devices of this invention will be principally of interest in identifying and quantifying protein in blood and blood components such as plasma and serum. It is recognized however, that the invention is also applicable to other body fluids such as lymph, spinal fluid, synovial fluid, urine and saliva and to body fluid components other than proteins.
- 15 For convenience, the invention will be described principally as applied to the analysis of blood.

#### SUMMARY OF THE INVENTION

- This invention provides a processes for identifying and quantitatively determining the amount of a selected protein in a sample of body fluid containing the protein together with contaminating products such as proteins. The process is applicable, for example, and is particularly useful, for determining the presence and amount of LDL in blood in the presence of contaminants such as very low density lipoproteins (VLDL) intermediate density lipoproteins (IDL), HDL, and others.

- 25 In accordance with the invention, the body sample to be analyzed is passed through a series of laminated membranes which may be considered as two separate membrane units. Initially the units are not in contact with each other, but at the appropriate time they are brought into contact. This arrangement permits control

of the flow of the body fluid through the laminate for reasons which will be discussed in detail below. The first unit comprises a separator membrane superimposed in capillary contact with a reactor membrane. The second unit comprises a collector membrane superimposed on a capture membrane or as an extension of the capture membrane. Selected antibodies are appropriately distributed throughout the membrane structure for reasons hereinafter explained.

A number of structures can be designed to conduct the process of this invention. The structure will include support means for the separate membrane units and means for bringing the units into contact when necessary to carry out the procedure of the invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a sketch showing the arrangement of the membranes of this invention.

Fig. 2 is a perspective view of a presently preferred test medical kit of the present invention, prior to assembly.

Fig. 3 is a perspective view of the test kit of Figure 2, after assembly.

Fig. 4 is a top plan view of the test kit of Figure 1, after assembly.

Fig. 5 is an exploded view of the test kit of Figure 1 showing the arrangement of its parts.

### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The processes and devices of this invention will be understood from the following discussions taken with the figures.

Fig. 1 is a sketch showing the arrangement of the membranes as they are employed to carry out the process of this invention. The membranes will always be in this or an equivalent configuration no matter how they are supported in a specific device. In the figure, 1 is an optional, coarse, filter membrane constructed of glass or other equivalent material. Its function is to assist in the removal of large particles such as red blood cells. It is not essential to the invention since in most instances the coarse particles can be removed by filtering the body fluid through the separator membrane 2.

The separator membrane 2 is a microporous, hydrophilic, asymmetric membrane the average pore size of which decreases from top to bottom. The cells are indicated in the figure as 3. The pore size at the top will generally be larger than the most coarse particle which is to be removed from the sample. Accordingly, the separator membrane will separate coarse particles by filtration. If the body fluid under analysis is blood, the body fluid passing into the reactor membrane 4 will be plasma containing all lipoproteins such as LDL and HDL and dissolved contaminants including carbohydrates such as glucose and proteins such as protein C.

The separator membrane 2 and the reactor membrane 4 comprise the first membrane unit. The membranes are in capillary contact, more specifically the bottom surface of the separator membrane is in capillary contact with the top surface of the reactor membrane.

"Capillary contact" means that the membranes are in sufficient contact, usually complete, laminar surface contact, so that the fluid in one membrane flows into

the adjacent membrane because of the influence of capillary forces induced by the hydrophilic properties of the membrane. In most instances, there will be complete contact throughout the total surface areas of the adjacent membranes maintained by the use of spot adhesives and contact pressure.

- 5 The function of the reactor membrane is to remove contaminating substances such as proteins which will interfere with the measurement to be performed. This is accomplished by immobilizing on the reaction membrane a sufficient number of selected antibodies so that substantially all of the contaminating materials react with them and thereby become immobilized or irreversibly bound to the reactor
- 10 membrane.

- If for example, blood is under analysis for LDL cholesterol content, the reactor membrane may carry any of a number of known antibodies to HDL, VLDL, IDL, cholesterol and the like. Many of types of these antibodies are known and readily available, some from commercial sources. These include, for example, anti
- 15 APOA1, A2, E, C, anti HDL and the like. There will be no antibody to LDL in the reactor membrane with the result that the plasma containing LDL is free to flow into the collector membrane.

The second membrane unit as shown comprises a collector membrane 5 superimposed and in capillary contact with a capture membrane 6.

- 20 The second membrane unit is not initially in contact with the bottom surface of the reactor membrane. This is to allow sufficient incubation time for the antibodies in the reactor membrane to react with and bind the contaminating substances. This is normally from about 5 to 15 minutes, preferably 8 to 12 minutes. A 10 minute incubation period is usually selected.
- 25 At the end of the incubation period, the top surface of the collector membrane 5 is brought into capillary contact with the bottom surface of the reactor membrane 4,



thus bringing them into position so that the fluid for analysis can flow by capillary attraction into the collector membrane 5 and from there into the capture membrane 6.

5 The fluid which passes from the reactor membrane 4 to the membrane 5 will be substantially free of contaminants and, if it is present, will contain the protein to be determined. The collector membrane 4 will contain an antibody, preferably monoclonal, to the protein to be determined, suitably in excess, so that all of the said protein will react with the antibody to form a reaction product.

10 Methods of binding antibodies to substrate such as the membranes employed in this invention are well known and need not be described here. These methods include procedures for blocking those sites on the membrane substrates which are not saturated with antibodies, thereby preventing undesirable side reactions.

The membranes employed in this invention are well known and readily available. They are hydrophilic, microporous membranes and, in the case of separator  
15 membrane 2, they are also asymmetric. Methods of preparing these membranes are described, for example in European Patent 261734 and U.S. Patent 5,240,862. This latter patent describes and claims a bilayer membrane structure which is especially useful as the first membrane unit of this invention. Typically, useful membranes are prepared from a mixture of hydrophilic and hydrophobic polymers.  
20 Polysulfones, polyether sulfones and polyetheramides are useful as hydrophobic polymers. Polyvinyl pyrrolidones are useful as hydrophilic polymers. Nitrocellulose polymers are also suitable.

The pore size in the separator membrane varies from about 20-30  $\mu\text{m}$  at the upper surface to from about 1.8 to 3.0  $\mu\text{m}$  at the lower surface. The average pore size  
25 in the other membranes is from about 3 to 10  $\mu\text{m}$ .

In the collector membrane 5, the LDL or other analyte to be determined will react with an epitope on the reversibly bound antibody to form a complex. In the usual case, the antibody will be labelled so that the product formed will be a protein/labelled antibody complex. Suitable labels are well known and include, for example, gold or carbon particles or any of a variety of fluorescent or luminescent enzymes.

The capture membrane 6 contains immobilized bound antibodies at spaced segments 7, 8 and 9. These antibodies which are normally identical are selected to react with a second epitope on the analyte or protein to be determined. When the protein/labelled antibody complex reacts with an immobilized antibody a detectable product is formed in accordance with known art. Preferably, the detectable product is one which is visible to the naked eye. However, it can be detectable by other means. For example, the label may be selected so that the product is fluorescent, luminescent or is made visible by other means under light ranging from ultraviolet to infra red light.

The concentration of bound antibodies in each of the segments 7 through 9 will, in the case of LDL be selected to indicate the amount of LDL in the sample. For humans, the concentrations of LDL conform to the following norms:

|    |                  |                       |
|----|------------------|-----------------------|
|    | Low              | 1-2.6 m mol/L         |
| 20 | Normal           | 2.6 - 3.4 m mol/L     |
|    | Border Line High | 3.4 - 3.9 m mol/L     |
|    | Very High        | 3.9 m mol/L or higher |

In the practice of the invention, if no segment becomes detectable it will be known that the concentration of LDL in the sample is no higher than 2.6 m mol/L. However, if all of the antibody in segment 7 reacts, but segment 8 remains undetected, it will be known that the concentration of LDL is greater than 2.6, but less than 3.4 m mol/L. Similar considerations apply to the other segments.

The capture membrane may contain a control segment 10. However, it is not necessary that it do so. The control segment will contain an antibody which reacts with an antigen normally present in the sample to be analyzed but one which will not interfere with the principal reactions of the invention. The reaction product  
5 should become detectable under the conditions of the invention. Hence, the bound antibody will normally be labelled. Other control reagents are possible. The control reaction is not necessarily an antigen/antibody reaction.

The process of this invention is applicable to very low volumes of body fluid. Only a few drops of blood or other body fluid, e.g. about 100 ul are necessary for  
10 the practice of this invention.

Since the invention is designed for quantitative determinations, it is necessary that the volume of material in certain of the membranes be known. Accordingly, the dimensions of the reactor membrane 4 will be selected so that it will hold all of the sample delivered to it through the separator membrane 2. Since the reactor  
15 membrane 4 is, initially, separated from the collector membrane 5, the former may become saturated with the filtered body fluid under analysis, but the body fluid will not escape from the reactor membrane 4.

When the reactor membrane 4 is subsequently moved into capillary contact with the collector membrane 5, flow will continue as a result of capillary forces until a  
20 known volume of the sample is in the collector membrane 5 where the analyte reacts with the reversibly bound antibody to form the protein/labelled antibody complex. At that point, the first membrane unit is removed and the sample fluid is forced from the collector membrane 5 into the capture membrane 6 by the addition of a reaction inert washing fluid such as a buffer, for example a  
25 phosphate buffer.

The buffer is added in excess and, as a result, the fluid under analysis will flow through the elongated capture membrane 6 where the protein/labelled antibody

complex, if present will react with the pre-spaced immobilized antibodies as explained above. Detection of the reaction provides a quantitative measure of the amount of analyte in the body fluid.

While the process has been principally described for the determination of LDL, it  
5 will be readily apparent that it is applicable to the determination of other materials by selection of antibodies which are immobilized on the reactor membrane so that the only product to be determined passes through to the collector membrane.

A number of devices are possible for the practice of this invention. All will  
comprise a unitary structure for initially retaining a first membrane unit in  
10 proximity to but out of contact with a second membrane unit and, after a selected time interval which will permit the contaminating substance to be bound to the reactor membrane, the first membrane unit is brought into contact with the second membrane unit. The first membrane unit is removed to permit a washing fluid to be passed through the collector membrane and the capture membrane to permit the  
15 flow of the body fluid to be analyzed through these membranes for the purposes described above. The structures and function of the membranes comprising each membrane unit have also been described above.

Figs 2 through 5 illustrate the presently preferred device for practicing the process of this invention.

20 As shown in the figures, the device includes a bottom member 11 which is rectangular in plan view. The bottom member 11, and the other structural members may be produced from a number of commercially available materials, preferably "ADS" (acrylo-nitrile-butadiene-styrene trimer or polypropylene or polycarbonate.)

25 The bottom member 11 has a flat base 12 and an upstanding flange 13 which runs along the edge of the base 12 on its four sides. An elongated internal flange 14

forms an elongated rectangular cavity 15 for holding the second membrane unit 20 of the invention including the collector membrane 5 and the capture membrane 6. A suitable size for the base is 0.5-inches high, 2.0 inches wide and 3.5 inches long.

- 5 A cover member 25 is adapted to fit over the second membrane unit 20 and is secured on the bottom member 11. The cover member 25 has an arcuate end 26, an arcuate body portion 27, which may be rectangular, and two depressions 28A and 28B formed between the end 26 and the body portion 27.

The cover member 25 has a first elongated opening 29 adapted to receive a  
10 selected volume of the patients's blood or other sample to be analyzed. It has rounded ends. A second elongated opening 30 (display window), which may be rectangular, within the cover member 25, permits the physician or other medical personnel, to view the detectable product formed by reaction between a protein/labelled antibody complex and a capture antibody.

- 15 A snap-in member 35 is adapted to be removably connected within the depressions 28 and 29. The snap-in member 35 has an elongated opening 36 which is of the same size and shape as the opening 29 of the cover member 25. An elongated funnel member 40 has a hollow nozzle portion 41 which fits through the opening 36. An externally projecting rim 42 of the funnel member 40 mates with the top  
20 face 37 of the snap-in member 35 and prevents the funnel member from passing through the opening 36. The first membrane unit 45 is positioned at the bottom of funnel 40 and is held by the tapered shape of the funnel nozzle portion 41 or is adhered thereto.

The taper of the funnel nozzle 41 is selected so that, initially, the funnel 41 sits  
25 loosely in the snap in member 35 so that the first member unit 45 is held in proximity to, but out of contact with the second membrane unit 20. At the end of the incubation period the funnel 41 is pushed further into the snap in member 35

so that the membrane units 20 and 45 are superimposed in capillary contact. After the fluid has passed into the collector membrane 5, the snap in unit 35 containing the funnel 40 is removed and a sufficient quantity of washing liquid, suitably a phosphate or carbonate buffer is passed through the opening 29 onto the upper  
5 surface of the collector membrane 5 to wash all of the body sample into the capture membrane 6 wherein it moves by capillary action from the upstream end 42 towards the downstream end 43 so that the contained protein to be analyzed, if any is present as a protein/labelled antibody complex, will react with the capture antibody to form a detectable product. The number of products formed will be a  
10 measure of the quantity of detectable protein in the sample.

Other devices for the practice of the invention can be readily constructed by the skilled artisan. In fact, many variation of the invention are possibly without departing from its concept and scope. For example, the collector membrane is not necessarily separate from the capture membrane. It may be merely an extension  
15 thereof containing the labelled reversibly bound antibody to the protein to be determined. In that structure, the protein/labelled antibody complex forms upstream of the immobilized antibodies in the capture membrane and the second membrane unit of the invention in one element including a collector section and a capture section.

## WHAT IS CLAIMED IS

1. A device useful for the quantitative determination of a selected protein in a body fluid which contains the selected protein in admixture with other contaminating proteins which structure comprises two separate membrane units, the first unit comprising a removable separator membrane superimposed and in capillary contact with a reactor membrane, the second unit comprising a collector membrane superimposed in capillary contact with a capture membrane, means for initially maintaining the two units out of contact with each other and means for bringing the reactor membrane into capillary contact with the collector membrane after a selected time interval; the membranes being characterized as follows:

A: the separator membrane is a porous hydrophilic, asymmetric membrane the average cell size of which decreases from top to bottom;

B: the reactor membrane is a porous hydrophilic membrane to which antibodies reactant with the contaminants but not with the protein to be determined are immobilized, the dimensions of the membrane being selected so that the membrane is capable of retaining the volume of body fluid which passes into it from the separator membrane;

C: the collector membrane is a porous membrane to which a labelled antibody which will react with the protein to be determined to form a protein/labelled antibody complex is reversibly bound;

D: the capture membrane is an elongated, porous, hydrophilic membrane having an upstream end in capillary contact with the collector membrane and a downstream end and having spaced apart segments, each segment containing a selected concentration of an immobilized capture antibody which will react with the protein/labelled antibody complex to form a detectable product.

2. A process for determining the amount of a selected protein in a sample of body fluid containing said protein in admixture with contaminants utilizing a detection device comprising two separator membrane units, the first unit comprising a separator membrane superimposed in capillary contact with a reactor  
5 membrane, the second unit comprising a collector membrane superimposed in capillary contact with a capture membrane, there being initially no contact between the units; the membranes being characterized as follows:

A: the separator membrane is a porous, hydrophilic, asymmetric membrane the average cell size of which decreases from top to bottom;

- 10 B: the reactor membrane is a porous hydrophilic membrane to which antibodies reactant with the contaminants but not with the proteins to be determined are immobilized, the dimensions of the membrane being selected so that the membrane is capable of retaining the volume of body fluid which passes into it from the separator membrane;

- 15 C: the collector membrane is a porous membrane to which a labelled antibody which will react with the protein to be determined to form a protein/labelled antibody complex is reversibly bound;

- D: the capture membrane is an elongated, porous, hydrophilic membrane having an upstream end in capillary contact with the collector  
20 membrane and a downstream end and having spread apart segments, each segment containing an immobilized capture antibody which will react with the protein/labelled antibody complex to form a detectable product;

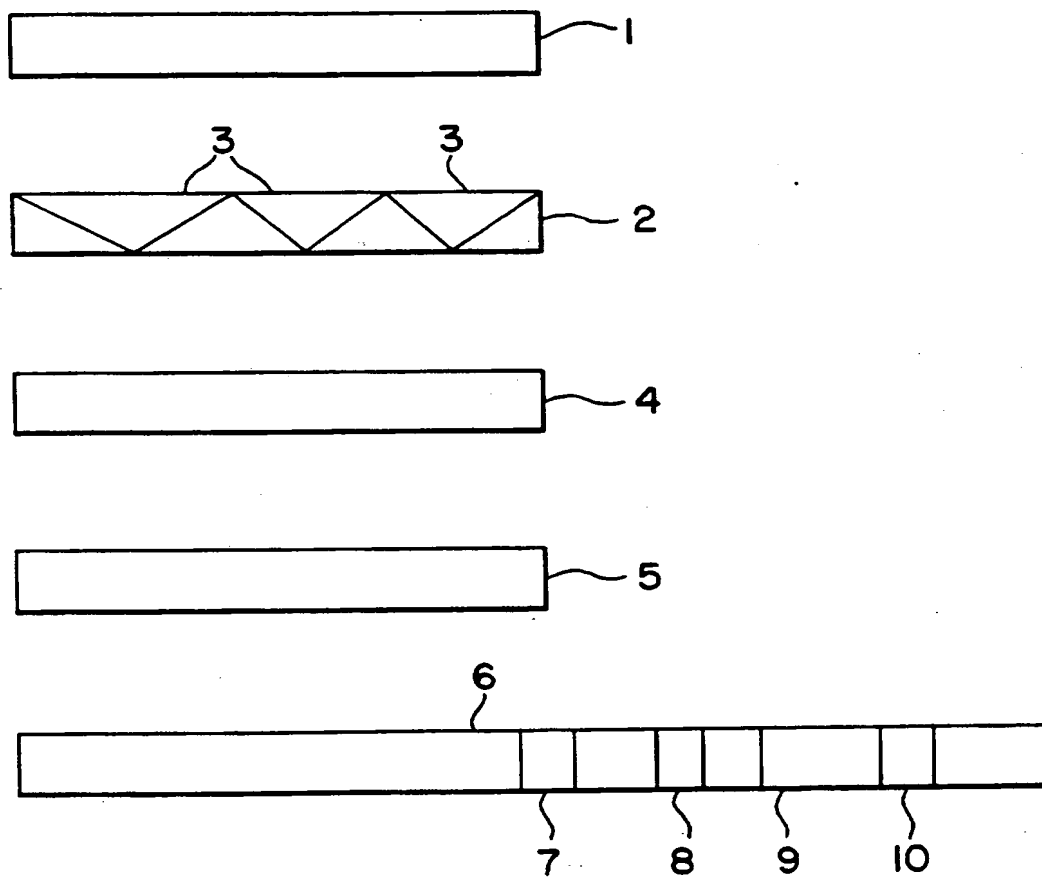
said process comprising the steps of:



1. filtering a selected volume of body fluid through the separator membrane and into the reactor membrane to retain large particles in the separator membrane;
2. retaining the filtered body fluid in the reactor membrane to permit  
5 the contaminants to react with the antibodies and become immobilized;
3. bringing the collector membrane into capillary contact with the separator membrane to permit a known volume of the body fluid containing the protein to be determined to flow from the reactor membrane into the collector membrane to permit the protein to be determined to react with the labelled  
10 antibody to form a protein/labelled antibody complex;
4. removing the first membrane unit from capillary contact with the collector membrane and adding washing liquid to the collector membrane to wash the body fluid containing the protein/labelled antibody complex into the capture membrane at its upstream end and to flow by capillary flow to the downstream end  
15 thereby permitting the protein/labelled antibody complex to react with the immobilized antibody in each successive segment so long as the filtered body fluid contains protein/labelled antibody complex and determining the amount of the protein to be determined which is in the body fluid by reading the detectable product.
- 20 5. A process as in claim 2 wherein the body fluid is whole blood and the protein to be determined is low density lipoprotein.

1 / 3

FIG. 1



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FIG.2

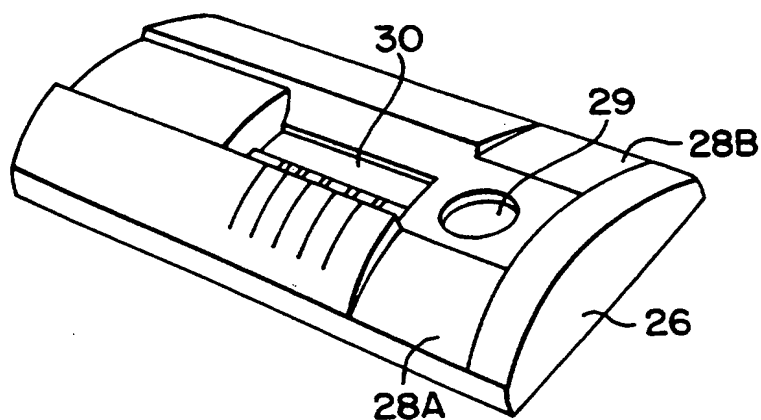


FIG.3

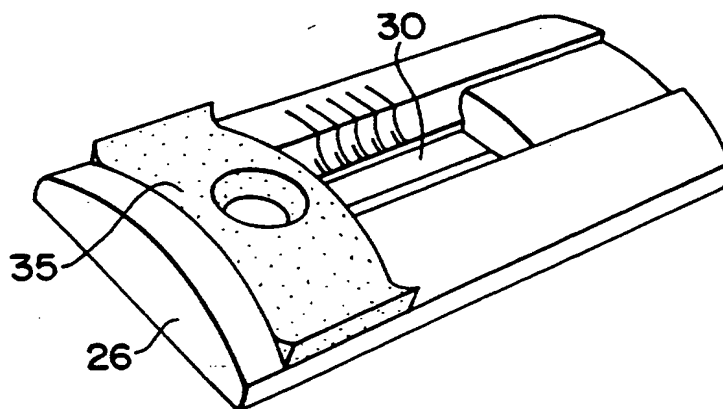
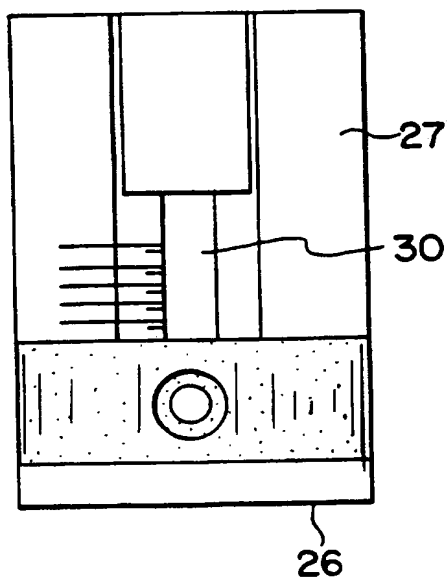
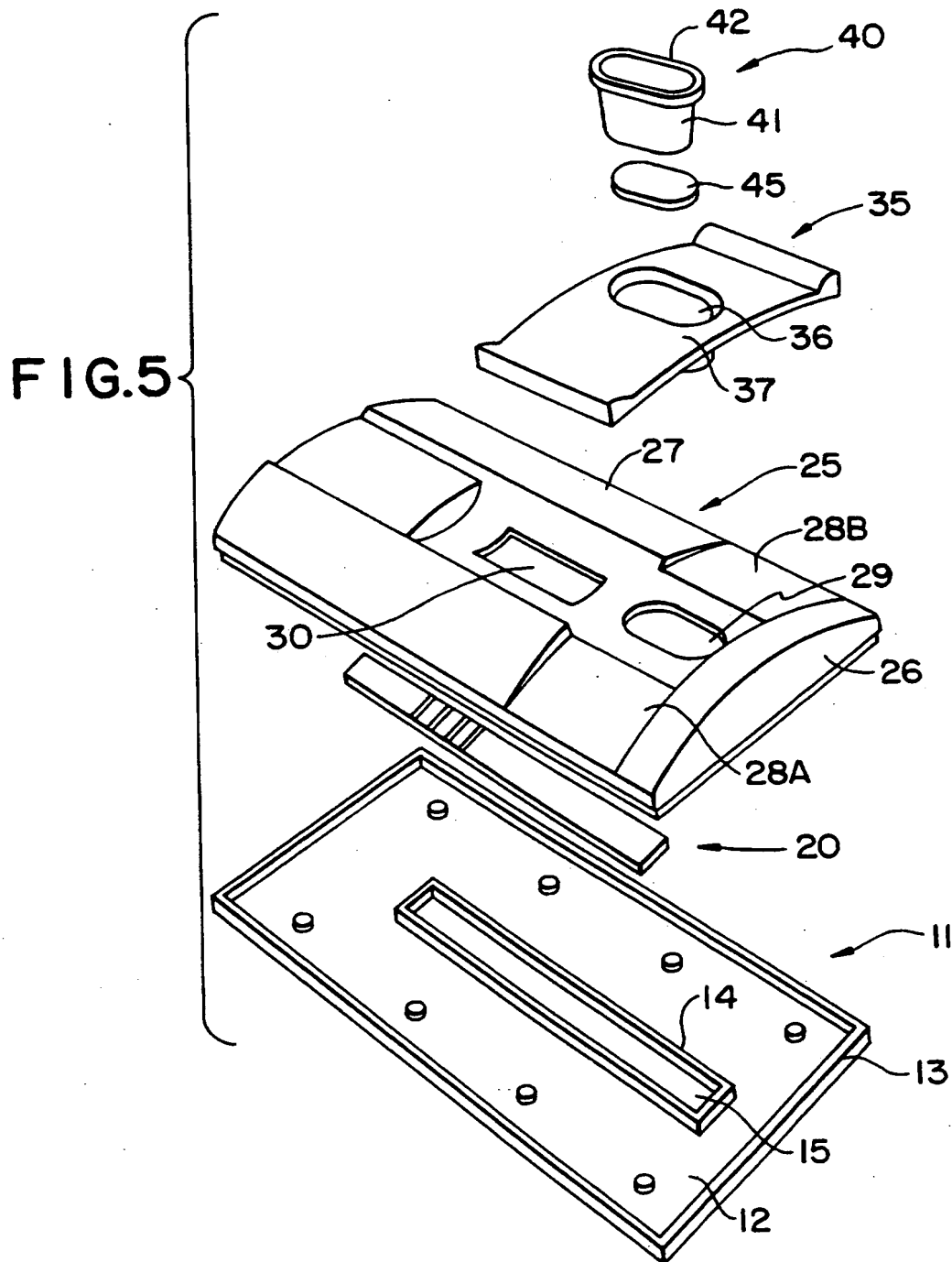


FIG.4



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## INTERNATIONAL SEARCH REPORT

International Application No

PC 1, IB 95/00999

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/543 G01N33/558 G01N33/68 G01N33/92

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                       | Relevant to claim No. |
|------------|--|-----------------------|
| A          | EP,A,0 525 829 (BOEHRINGER MANNHEIM GMBH)<br>3 February 1993<br>---  |                       |
| A          | WO,A,92 01226 (UNILEVER PLC ;UNILEVER NV<br>(NL)) 23 January 1992<br>---   |                       |
| A          | WO,A,91 17441 (GENZYME CORP) 14 November<br>1991<br>---  |                       |
| A          | EP,A,0 597 268 (MILES INC) 18 May 1994<br>---  |                       |
| A          | EP,A,0 336 483 (X FLOW BV ;PRIMECARE B V<br>(NL)) 11 October 1989<br>cited in the application<br>& US,A,5 240 862<br>--- |                       |
| P,A        | EP,A,0 627 627 (MILES INC) 7 December 1994<br>-----  |                       |

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Date of the actual completion of the international search

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Name and mailing address of the ISA

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| EP-A-0525829                              | 03-02-93            | DE-A-                      | 3842702             | 21-06-90 |
|   |                     | AU-B-                      | 616328              | 24-10-91 |
|   |                     | AU-B-                      | 4616889             | 21-06-90 |
|   |                     | CA-A-                      | 2005564             | 19-06-90 |
|   |                     | EP-A,B                     | 0374684             | 27-06-90 |
|   |                     | JP-A-                      | 2221860             | 04-09-90 |
|   |                     | US-A-                      | 5160486             | 03-11-92 |
| -----                                     |                     |                            |                     |          |
| WO-A-9201226                              | 23-01-92            | AU-B-                      | 7903694             | 04-05-95 |
|   |                     | AU-B-                      | 656528              | 09-02-95 |
|   |                     | AU-B-                      | 8101591             | 04-02-92 |
|   |                     | EP-A-                      | 0491024             | 24-06-92 |
|   |                     | JP-T-                      | 4507146             | 10-12-92 |
| -----                                     |                     |                            |                     |          |
| WO-A-9117441                              | 14-11-91            | US-A-                      | 5403745             | 04-04-95 |
|   |                     | AU-B-                      | 659797              | 01-06-95 |
|   |                     | AU-B-                      | 7762491             | 27-11-91 |
|   |                     | CA-A-                      | 2081559             | 28-10-91 |
|   |                     | EP-A-                      | 0527212             | 17-02-93 |
| -----                                     |                     |                            |                     |          |
| EP-A-0597268                              | 18-05-94            | US-A-                      | 5460974             | 24-10-95 |
|   |                     | AU-B-                      | 661414              | 20-07-95 |
|   |                     | AU-B-                      | 4898893             | 04-08-94 |
|   |                     | CA-A-                      | 2108105             | 14-04-94 |
|   |                     | JP-A-                      | 6201704             | 22-07-94 |
| -----                                     |                     |                            |                     |          |
| EP-A-0336483                              | 11-10-89            | NL-A-                      | 8800796             | 16-10-89 |
|   |                     | AT-T-                      | 108021              | 15-07-94 |
|   |                     | CA-A-                      | 1322335             | 21-09-93 |
|   |                     | DE-D-                      | 68916458            | 04-08-94 |
|   |                     | DE-T-                      | 68916458            | 01-12-94 |
|   |                     | ES-T-                      | 2058470             | 01-11-94 |
|   |                     | JP-A-                      | 1302161             | 06-12-89 |
|   |                     | US-A-                      | 5240862             | 31-08-93 |
|   |                     | -----                      |                     |          |
| EP-A-0627627                              | 07-12-94            | US-A-                      | 5401466             | 28-03-95 |
|   |                     | AU-B-                      | 6311694             | 08-12-94 |
|   |                     | JP-A-                      | 7055812             | 03-03-95 |
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